August 16, 1956

Dr. Louis Dienes
Department of Pathology
Massachusetts General Hospital
Boston 14, Mass.

Dear Dr. Dienes:

Among the enclosed reprints, I believe you may be specially interested in the preprint on penicillin-induced protoplasts. It is of course obvious that this finding is a direct extension of your own work, differing primarily in its emphasis of quantitative and enzymatic relations.

I have not, over the last several years since my visit with you, lost interest in I-forms, and this recent work has only renewed my active concern over them. I have not, however, succeeded in preliminary attempts to secure further outgrowth of protoplasts as such, i.e., they are fully viable, and can increase tremendously in size, but have not been observed to divide except as they revert to rods. A plausible view of large bodies on this basis might be that they represent either mutants, or a penicillin-inhibited physiological state, inwhich wall synthesis is largely, but not completely inhibited, and our further efforts will be directed along this working hypothesis.

My immediate aim would be to secure the outgrowth of protoplasts as I-type growth in E. coli K-12, but to do this it seems important for me to regain some familiarity with I-growth in more favorable material. I wish I had been able to maintain the I-form of Proteus 52, which I took back from your laboratory in November 1953, and which I did succeed in propagating according to your instructions. I do have the lyophilized cultures of this Proteus, however, and have begun to revive them.

As I recall both from my own experience, and your advice, the recently prepared L-form from Proteus 52 has a marked tendency to revert in the absence of penicillin. It might save me a considerable amount of time for some of these studies if I could also deal with the stabilized form, such as you gave me a subculture of in 1953. Have you maintained this? Would it be feasible to send it to me? (This is the one which was propagated by flotation on the surface of broth; as I recall, however, it would also grow reasonably well on nutrient-gelatin-agar (motality agar)).

You have also mentioned the propagation of Salmonella typhimurium L-forms in thioglycollate broth. This suggests the most premising approach for my own problem; do you have any cultures growing in this medium now that you could send me?

Finally, I have to admit that I have never studied wither S. moniliformis or PPLO forms as make such. Could I impose upon you for cultures of these too.

This is a large order. I am asking you for

- 1. stabilized L forms from Proteus 52 (flotation form)
- 2. L-forms of S. typhimurium, growing in Brewer's medium
- 3. S. moniliformis (capable of transformation into L_1)
- 4. Any "typical" PPLO.

I would not ask such an imposition if I did not already respect your generosity, but for the same reason please to not go to any undue trouble if you are not maintaining these materials at ready hand.

With best regards,

Yours sincerely,

Joshua Lederberg
Professor of Genetius